IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Pergolizzi, et al.	Group Art Unit:	1631
Application No.:	08/479,995	Examiner:	Ardin H. Marschel
Filed:	June 7, 1995	Att'y Dkt. No.:	ENZ-11(C2)(D1)(C2)
Title:	ANALYTE DETECTION UTILIZING POLYNUCLEOTIDE SEQUENCES, COMPOSITION, PROCESS AND KIT		

SECOND REQUEST FOR AN INTERFERENCE PURSUANT TO 37 C.F.R. § 1.607

Director of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

On December 20, 2002, the above-named Applicants (hereafter "Pergolizzi") filed a first Request for an Interference in the above-named Application ("the Pergolizzi Application"). This Second Request involves the same patents but proposes different counts and discusses different claims from the Pergolizzi Application. Please disregard the first Request and consider this Second Request instead.

Over five years ago, Pergolizzi informed the Examiner that certain patents appear to interfere with the Pergolizzi Application.[1] At the time, Pergolizzi did not file a formal request for an interference, but three years ago the Examiner suspended prosecution of the Pergolizzi Application in light of the apparent interference.[2] Prosecution has remained suspended though no interference has been declared yet. Pergolizzi now respectfully requests prompt declaration of an interference between (1) certain claims of the Pergolizzi Application and (2) certain claims of U.S. Patent Nos. 4,716,106; 4,882,269; 5,424,188; and 5,124,246.

Immediately below is an Executive Summary of the Invention. Below the Executive Summary is the information required by 37 C.F.R. § 1.607(a), under headings that correspond to the

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[1] On pages 29-31 and 80-84 of Pergolizzi's Amendment of July 25, 1997, Pergolizzi reproduced and discussed claims from U.S. Patent Nos. 4,716,106, 4,868,105, and 4,882,269. A potential interference with these patents was also discussed at the Interview of October 29, 1998. *See* Interview Summary of October 29, 1998.

[2] Prosecution was suspended pursuant to the Office Communications of March 14, 2000, April 12, 2000, March 26, 2001, and March 19, 2002.

six subsections of Rule 1.607(a). The attached Appendices 1-6 also correspond to these six subsections.

EXECUTIVE SUMMARY OF THE INVENTION

The Pergolizzi Application concerns the enhanced detection of minute substances in biological or non-biological samples. More specifically, the claimed invention provides a more sensitive, efficient, and universal means for detecting analytes with nucleic acid or immuno assays.[3] The Pergolizzi Application is entitled to a priority filing date of May 5, 1983.

In both the invention and the prior art, assays entail two fundamental events: recognition of the analyte and signaling of that recognition. For nucleic acid assays, recognizing the analyte means a nucleic acid sequence, often called a "detection probe," binds to a complementary nucleic acid sequence of the analyte.[4]

Signaling is often divided between radioactive signaling, which relies on radioisotopes such as ^{32}p, and non-radioactive signaling, which relies on techniques such as fluorescence or avidin-biotin linkage.[5] In the prior art, signaling typically employs radioisotopes, which are dangerous, difficult to handle, and whose short half-lives necessitate continuous production of new probes.

In both the invention and the prior art, the recognition event must be coupled with the signaling event.[6] However, the invention's means of coupling diverges from that of the prior art. In the prior art, the recognition probe and the signal probe both bind to the analyte.[7] Either they bind sequentially or, when the recognition and signal probe are one and the same thing, they bind simultaneously. The recognition and signal probe are one and the same in generic nucleic acid assays that employ "labeled detection probes." Thus, in generic nucleic acid assays the recognition event (detection) is inherently coupled to the signaling event because the labeled detection probes both recognize the analyte and signal that recognition.

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[3] *See, e.g.*, Pergolizzi Application (p. 7).

[4] *Id.* at 1-2.

[5] *Id.* at 2-3, 19-21.

[6] *Id.* at 2-3.

[7] *Id.* at 3-4.

Generic nucleic acid assays have various disadvantages. Labeling a polynucleotide chain chemically is problematic because it is difficult to selectively label individual nucleotide residues in the chain.[8] Chemical-based procedures require reaction conditions that are generally too vigorous to limit modification to just one or a few nucleotide residues. Dicarbonyl reagents, for example, react indiscriminately with guanine residues, thereby impairing the chain's ability to recognize (bind to) the analyte.[9] To overcome these problems, researchers must first synthesize individual modified nucleotides, which often involves elaborate procedures. Then the researchers must incorporate the modified nucleotides into the chain using enzymatic techniques.[10] Although incorporation using enzymes diminishes the conflict between a probe's signaling function and its recognition function, it does not erase the conflict, particularly if the researcher wants to amplify the signal via a high ratio of signal groups to recognition groups.[11]

In another type of prior art assay, "classic sandwich hybridization," a capture probe binds to one side of the analyte (recognition event) and a separate signal probe binds to the other side of the analyte (signaling event or a precursor thereof), thereby coupling the recognition and signaling events as the analyte is sandwiched between the two probes. A disadvantage of sandwich hybridization is that each unique analyte requires independent synthesis of not only a unique recognition probe but also a unique signal probe—both of which must be complementary to adjacent non-overlapping sections of the target analyte.

The invention overcomes these disadvantages by (1) making the signal probe bind to the recognition probe rather than to the analyte and (2) including at least two distinct portions on the recognition probe—a first portion that binds to the analyte and a second portion to which the signal probes bind.[12] In other words, the recognition probe serves as bridge between the analyte and the signal probe. This bridge obviates the conflict in the prior art between efficient synthesis of signal

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[8] *Id.* at 5.

[9] *Id.* at 5.

[10] *Id.* at 5-6, 24-25.

[11] *Id.* at 4, 6, 22.

[12] *Id.* at 7-9.

probes and the specificity of those signal probes for the analyte. This bridge also dramatically reduces the need to synthesize unique signal probes—because the same signal probes can be used for any assay. In other words, since the second portion of the recognition probe can remain the same, the complementary sequence of the signal probe can remain the same.[13]

The interfering patents discussed below are directed to substantially the same invention as the Pergolizzi Application. These patents identify substantially the same obstacles in the prior art and they propose substantially the same means of overcoming those obstacles.[14] The interfering

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[13] *Id.* at 8-9, 24-25.

[14] For example, consider the following passage from interfering U.S. Patent No. 4,716,106:

> All these prior workers used a labelled single-stranded polynucleotide probe to hybridise with the target sequence. Each probe for each different assay has to be labelled separately. Labelling of the probe necessarily involves an additional preparative step, and one which may in some cases be difficult. The present invention seeks to avoid this problem by the use of a labelled secondary probe which does not have to be complementary to the target sequence and can therefore be used in assays for a variety of different targets. '106 Patent, col. 2, lines 1-10 [emphasis added].

Consider also the following two passages, which appear in both interfering U.S. Patent No. 4,882,269 and interfering U.S. Patent No. 5,424,188:

> However, there are several disadvantages to these methods [of nucleic acid hybridization]. First, the production of probes requires the use of radioactive isotopes which have short half-lives necessitating a continuous production of fresh probes. Second, the labeling procedure requires the use of enzymes which are expensive and require reaction conditions which must be very carefully calibrated. Third, radioactive isotopes are biologically dangerous to use.... '269 Patent, col. 2, lines 23-31; '188 Patent, col. 2, lines 27-35 [emphasis added].
>
> Another problem encountered is that the alteration of the nucleotides interferes with hybridization of the probe to its target. '269 Patent, col. 2, lines 47-49; '188 Patent, col. 2, lines 51-53 [emphasis added]. *See also* '269 Patent, col. 7, lines 18-22; '188 Patent, col. 7, lines 23-27.

Finally, consider the following two passages from interfering US Patent No. 5,124,246:

> A primary object of the present invention is to provide an amplifier for use in nucleic acid hybridizations that provides a high reproducible gain in signal...and that is capable of combining specifically with a "universal" signal moiety and an analyte at

(continued...)

patents assert priority to 1984, 1985 or 1987. Again, the Pergolizzi Application is entitled to a priority filing date of May 5, 1983, and it has no continuation-in-part in its ancestral line.

REQUIREMENTS OF 37 C.F.R. § 1.607(a)

(1) Identification of Patents that Interfere with Application (37 C.F.R. § 1.607(a)(1))

The following information is reproduced in Appendix 1.

A. U.S. Patent No. 4,716,106

The '106 Patent issued on December 29, 1987, to David J. Chiswell for "Detecting Polynucleotide Sequences." It issued from Application No. 706,747, filed February 28, 1985, which claims the benefit of a British application filed March 1, 1984. Amersham International is the assignee named on the face of the '106 Patent. Pergolizzi first informed the Examiner that the '106 Patent interferes with the Pergolizzi Application in Pergolizzi's Amendment of July 25, 1997.

B. U.S. Patent No. 4,882,269

The '269 Patent issued on November 21, 1989, to Robert J. Schneider and Thomas E. Shenk for an "Amplified Hybridization Assay." It issued from Application No. 06/940,712, filed December 11, 1986, which is a continuation-in-part of Application No. 06/808,695, filed December 13, 1985. Princeton University is the assignee named on the face of the '269 Patent. Pergolizzi first informed the Examiner that the '269 Patent interferes with the Pergolizzi Application in Pergolizzi's Amendment of July 25, 1997.

> low concentrations to form a stable complex. '246 Patent, col. 2, lines 6-13 [emphasis added].
>
> By using an amplifier probe, the multimer may be designed to be a "universal" reagent and different multimers need not be made for each analyte. '246 Patent, col. 14, lines 39-41 [emphasis added].

Note that the '246 Patent uses terms inconsistently. In some claims of the '246 Patent, the "multimer" corresponds to the Pergolizzi signalling entity and the "amplifier probe" corresponds to the Pergolizzi bridging entity. In other '246 patent claims, the multimer corresponds to the Pergolizzi bridging entity and the amplifier probe is not recited.

C. U.S. Patent No. 5,424,188

The '188 Patent issued on June 13, 1995, to Robert J. Schneider and Thomas E. Shenk for an "Amplified Hybridization Assay." It issued from Application No. 07/963,923, filed October 20, 1992, which is a continuation of Application No. 07/400,831, filed August 29, 1989, which is a divisional of Application No. 06/940,712 (the '269 Patent), filed December 11, 1986, which is a continuation-in-part of Application No. 06/808,695, filed December 13, 1985. Princeton University is the assignee named on the face of the '188 Patent. The '188 Patent is terminally disclaimed over the '269 Patent.

D. US Patent No. 5,124,246

The '246 Patent issued on June 23, 1992, to Michael S. Urdea, Brian Warner, and Thomas Horn for "Nucleic Acid Multimers and Amplified Nucleic Acid Hybridization Assays Using Same." The '246 Patent issued from Application No. 340,031, filed April 18, 1989, which is a continuation-in-part of Application No. 252,638, filed September 30, 1988, which is a continuation-in-part of Application No. 185,201, filed April 22, 1988, which is a continuation-in-part of Application No. 109,282, filed October 15, 1987. Chiron Corporation is the assignee named on the face of the '246 Patent. Pergolizzi first apprised the Examiner of the existence of the '246 Patent in Pergolizzi's Amendment of March 5, 1996.[15]

(2) Presentation of Proposed Counts (37 C.F.R. § 1.607(a)(2))

Proposed Count 1

Proposed Count 1 is identical to Claim 1 of the '106 patent. It appears below and in Appendix 2.

A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of

(a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and

[15] *See also* Pergolizzi's Amendment of July 25, 1997 (p.57).

(b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of

(i) contacting the sample under hybridisation conditions with the primary probe,

(ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and

(iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.

Proposed Count 2

Proposed Count 2 is identical to Claim 1 of the '269 patent. It appears below and in Appendix 2.

A method for the detection of a target nucleotide sequence, comprising:

(a) contacting the target nucleotide under conditions that permit hybridization with

(i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and

(ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and

(b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.

Proposed Count 3

Proposed Count 3 is identical to Claim 25 of the '269 patent. It appears below and in Appendix 2.

A hybridization assay kit for the detection of a target nucleotide sequence, comprising:

(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and

(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.

Proposed Count 4

Proposed Count 4 is substantively identical to Claim 53 of the '246 patent. Claim 53 depends from Claim 51, which depends from Claim 39. Proposed Count 4 is simply Claim 53 with the limitations of Claims 39 and 51 explicitly reproduced in it. It appears below and in Appendix 2.

A nucleic acid hybridization assay wherein:

I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:

(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and

(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;

II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;

III. unbound multimer is removed;

IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;

V. unbound labeled oligonucleotide is removed; and

VI. the presence of label bound to the multimer is detected.

(3) Claims in Patents that Correspond to Counts (37 C.F.R. § 1.607(a)(3))

A. Count 1 (Claim 1 of '106 Patent)

Claim 1 of the '106 Patent is identical to Count 1 and therefore corresponds exactly to Count 1. Claims 2-10 of the '106 Patent correspond substantially to Count 1 because they are patentably indistinct from Count 1. In other words, if Count 1 were prior art to Claims 2-10 of the '106 Patent, Count 1 would anticipate or render them obvious.[16]

Claim 2 simply recites that the sample is immobilized. Claim 3 recites that the sample is contacted by the primary probe before the secondary probe. Claim 4 recites that the primary and secondary probe are mixed before contact with the immobilized sample. These variations are self-evidently obvious and were well-known in the art. Claims 5-7 and 9 recite that the primary or secondary probes are derived from a vector, *i.e.*, they are cloned. Cloning probes in conjunction with their use in assays is also obvious and was well-known in the art. Claim 8 is vague but appears to recite that the primary probe is single-stranded when it first contacts the sample, then partially double-stranded hybrids are formed between the primary probe and the sample, and then single-stranded secondary probes are added. This variation is also self-evidently obvious, if not inherent in Claim 1. Claim 10 recites that the primary probe is DNA and that the secondary probe is RNA. Forming RNA-DNA hybrids between probes was also obvious and well-known in this art and likewise does not render the claim patentably distinct from Count 1.

B. Count 2 (Claim 1 of '269 Patent)

Claim 1 of the '269 Patent is identical to Count 2 and therefore corresponds exactly to Count 2. Dependent Claims 2-24 of the '269 Patent correspond substantially to Count 2 because they are patentably indistinct from Count 2. In other words, the features added by Claims 2-24 are

[16] The "test for claim correspondence is whether the count anticipates or renders obvious the claim." Judge Michael Tierney, 'Preliminary Tasks in Declaring the Interference,' in tab H of handbook for IPO conference *PTO Appellate and Interference Practice in the New Millennium* (September 2002). The test is not whether the claim anticipates or renders obvious the count.

obvious in view of Count 2. Indeed, in the Background section of the '269 Patent (col. 2-4) most of these added features are disclosed as being in the prior art.

C. Count 3 (Claim 25 of '269 Patent)

(i) Claims 25-48 of '269 Patent:

Independent Claim 25 of the '269 Patent is identical to Count 3 and therefore corresponds exactly to Count 3. Dependent Claims 26-48 of the '269 Patent correspond substantially to Count 3 because the features they recite are obvious in view of Count 3. In the Background section of the '269 Patent (col. 2-4), most of the features recited by Claims 26-48 are disclosed as being in the prior art.

(ii) Claims 49-62 of '269 Patent:

Independent Claim 49 of the '269 Patent corresponds substantially to Count 3 because the features recited by Claim 49 are obvious in view of Count 3. A side-by-side comparison of Count 3 and Claim 49 appears below and in Appendix 3.

Count 3 (Claim 25 of US4882269)	**Claim 49 of US4882269**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.	A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe cassette which comprises a cloning vector having (i) a multiple cloning site into which a target nucleotide sequence can be inserted and cloned and (ii) nucleotide sequences which are capable of hybridizing to their complements which comprise a plurality of secondary probes; and (b) the plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a nucleotide sequence capable of hybridizing to a different portion of the portion of the primary probe described in (a)(ii), which provides for the generation of an amplified signal when the plurality of secondary probes are hybridized to different portions of the portion of the primary probe described in (a)(ii).

Two features in Claim 49 above are not explicitly recited in Count 3: (1) "primary probe cassette which comprises a cloning vector" and (2) "multiple cloning site into which a target nucleotide sequence can be inserted and cloned." These features are obvious in view of the primary probes of Count 3. Cassette and vector type polynucleotides are the immediate precursors of detection probes and have long been used to clone detection probes for use in assays.[17]

The features recited by dependent Claims 50-62 are also obvious in view of Count 3. Again, in the Background section of the '269 Patent (col. 2-4), most of these features are disclosed as being in the prior art.

(iii) Claims 1-19 of '188 Patent:

On October 14, 1994, the applicants for the '188 Patent terminally disclaimed the '188 Patent over the '269 Patent. Thus, they acquiesced to the proposition that the claims of the '188 Patent are patentably indistinct from those of the '269 Patent. Furthermore, Claims 1-19 of the '188 Patent are in fact patentably indistinct from at least Claims 25-62 of the '269 Patent. A side-by-side comparison of Claim 1 of the '188 Patent and Count 3 (Claim 25 of the '269 Patent) appears below and in Appendix 3.

Count 3 (Claim 25 of US4882269)	**Claim 1 of US5424188**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and	A hybridization assay kit for the detection of a target nucleotide sequence in a sample which target is hybridized to a primary probe, which primary probe has (1) a polynucleotide sequence complementary to the target nucleotide sequence and (2) a polymeric tail with a plurality of

[17] As stated in the '269 Patent:

> The portion of the primary probe which is complementary to the target sequence…usually, but need not be, produced by cloning the target sequence into a recombinant vector such as a plasmid or virus which can be used to generate many copies of the sequence. Alternatively, the primary probes may be synthesized by chemical methods. These procedures are well known to one skilled in the art. '269 Patent, col. 8, lines 17-26. *See also* 269 Patent, col. 2, lines 57-63, col. 5, lines 52-57, col. 8, lines 43-57.

(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.	binding sites, each site incapable of binding to the target sequence and capable of binding a member of a family of secondary probes, which kit comprises: a plurality of secondary probes comprising a family of signal-generating probes, each member of the family having at least (1) a signal-generating component and (2) a polymer capable of binding to a distinct binding site of the tail of the primary probe which site is not bound by other members of the family; which kit provides for the generation of an amplified signal when the plurality of secondary probes are bound to distinct binding sites of the tail of the primary probe.

As shown above, Claims 1-19 of the '188 Patent are obvious in view of, and therefore correspond substantially to, Count 3. The features recited by dependent Claims 2-19 of the '188 Patent are also obvious in view of Count 3. In the Background section of the '188 Patent (col. 2-4), most of these features are disclosed as being in the prior art.

D. Count 4 (Claim 53 of '246 Patent)

(i) Claims 53-56 of the '246 Patent:

Dependent Claim 53 of the '246 Patent is substantively identical to Count 4 and therefore corresponds almost exactly to Count 4. Claims 54-56, which depend from Claim 53, correspond substantially to Count 4 because the features recited by Claims 54-56 are obvious in view of Count 4.

(ii) Claims 39-41 and 43-52 of the '246 Patent:

Claim 53 (Count 4) expressly incorporates the multimer of Claim 39.[18] Claim 53 is directed to an assay, whereas independent Claim 39 is directed to a multimer used in the assay of Claim 53. A side-by-side comparison of Claim 39 and Count 4 appears below and in Appendix 3.

[18] Claim 53 depends from Claim 51 which depends from Claim 39. Claim 53 starts as: "A nucleic acid hybridization assay wherein: (a) the multimer of claim 51 is...." In turn, Claim 51 (continued...)

Count 4 (Claim 53 of US5124246)	Claim 39 of US5124246
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	A synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds.

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reads: "The nucleic acid multimer of claim 39 wherein the second single-stranded nucleotide sequence of interest is a sequence of a single-stranded labeled oligonucleotide." Count 4 includes the relevant text of Claims 39, 51 and 53.

As shown above, Claim 39 is essentially identical to section I of Count 4. Although Claim 39 is directed to a multimer used in the assay rather than to the assay itself, Claim 39 corresponds substantially to Count 4.[19]

Claim 51 depends directly from independent Claim 39. The feature recited by Claim 51—the second sequence of interest is a single-stranded labeled oligonucleotide—is also recited in Section IV of Count 4. Claims 40-41 and 43-52, which also depend from Claim 39, recite features that are obvious in view of Count 4. Therefore, Claims 40-41 and 43-52 likewise correspond substantially to Count 4.

(4) Claims of Application that Correspond to Counts (37 C.F.R. § 1.607(a)(4))

A. Count 1 (Claim 1 of the '106 Patent)

Pergolizzi Claims 532-35 correspond substantially to Count 1. Side-by-side comparisons of Count 1 and Claims 532-35 appear below and in Appendix 4. Also appearing below and in Appendix 4 is Pergolizzi Claim 443, from which Claims 532-35 depend.

Count 1 (Claim 1 of the US4716106)	Claims 443 and 532-35 of Pergolizzi Application
A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of (a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and (b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of (i) contacting the sample under	443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising: providing a composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more signalling entities substantially incapable

[19] Again, the "test for claim correspondence is whether the count anticipates or renders obvious the claim." Judge Tierney, *supra* The test is not whether the claim anticipates or renders obvious the count. Although a count directed to a multimer may or may not render obvious a claim to an assay that uses the multimer, a count to an assay that uses the multimer should render obvious a claim to the multimer.

hybridisation conditions with the primary probe, (ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and (iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.	of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal; forming a complex comprising said composition and said analyte; and detecting said analyte by a signal provided by said signal generating portion or portions present in said complex. 532. The process of claim 443, wherein said analyte is a DNA sequence, said bridging entity is a single-stranded DNA sequence, and said signalling entities are single-stranded DNA sequences. 533. The process of claim 532, wherein said bridging entity is derived from a filamentous phage. 534. The process of claim 533, wherein said signalling entities are derived from filamentous phages. 535. The process of claim 534, wherein said bridging entity codes for a gene product or fragment thereof, and said forming step comprises either (i) contacting said analyte with said bridging entity to form a first complex and thereafter contacting said first complex with said signalling entities to form said detectable complex or (ii) contacting said bridging entity with said signalling entities to form a first complex and thereafter contacting said first complex with said analyte to form said detectable complex.

Discussed below are features shared by Count 1 and Pergolizzi Claims 532-35. Not discussed below are certain shared features whose explicit or inherent presence in Claims 532-35 is

immediately apparent. Note also that some of the features discussed below may represent variants that are obvious under 35 U.S.C. 103(a), even if they are not identified as such herein.[20]

Target Polynucleotide/Analyte. Pergolizzi Claim 532 explicitly identifies the analyte as a DNA sequence. Furthermore, in Pergolizzi Claim 443 the first portion of the bridging entity is capable of "hybridizing" to the analyte, clearly implying that the analyte can be a polynucleotide.

Single-Stranded Primary Probe/Bridging Entity. Pergolizzi Claim 532 explicitly recites that the bridging entity is single-stranded. Furthermore, single-strandedness is inherent because the bridging entity must be single-stranded or must become single-stranded to hybridize to the analyte or to the signalling entities.

Complex Primary Probe/Bridging Entity. As used in Count 1, "complex" means only that the primary probe is not a low-complexity sequence with uniform or highly repetitive residues. Naturally occurring DNA, for example, is complex.[21] The word 'complex' appears in Pergolizzi Claims 532-35 where it is used as a noun (as in "the complex") rather than as an adjective. Nevertheless, the concept of the adjective is present. In Claims 532-35 the bridging entity is complex because: (1) it is a DNA sequence, (2) it must be complex to bind to the complex sequence of the analyte, (3) it is derived from a filamentous phage such as M13, or (4) it encodes a gene product. With regard to (1), note that the term DNA sequence, as typically used in the art, denotes a complex polynucleotide. This point also applies to (2). With regard to (3), note that a DNA sequence derived from a phage (as in Claims 533-34) is not usually a low-complexity sequence with uniform or highly repetitive residues. When researchers need low-complexity sequences, they do not derive them from phages. Accordingly, the primary probe in dependent Claims 5, 6 and 9 of the '106 Patent is both complex and derived from a phage or vector. With regard to (4), note that a DNA sequence that encodes a gene product is the epitome of complex.

Single-Stranded Secondary Probe/Signalling Entities. Pergolizzi Claim 532 explicitly recites that the signalling entities are single-stranded DNA sequences. Furthermore, Furthermore, single-

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[20] To say that a particular feature of Count 1 represents an obvious variant is shorthand for saying that Count 1 would be obvious under 35 U.S.C. 103(a) in view of a prior art reference that discloses every feature of Count 1 except for the particular feature.

[21] *See, e.g.*, '106 Patent, col. 2, lines 39-43.

strandedness is inherent because the signalling entities must be single-stranded or must become single-stranded to hybridize to the bridging entity.

Complex Secondary Probe/ Signalling Entities. The signalling entities are complex either because: (1) they are DNA sequences,(2) they must be complex to bind to the complex sequence of the bridging entity second portion, or (3) they are derived from filamentous phages (as in Claim 534).

Before, During or After/ Variable Order of Forming Complex. This feature appears explicitly in Pergolizzi Claim 535, which recites that either (1) the analyte and bridging entity can first form a complex that in turn forms a second complex with the signalling entities or (2) the signalling entities and bridging entity can first form a complex that in turn forms a second complex with the analyte.

Accordingly, Pergolizzi Claims 532-35 correspond substantially to Count 1.

B. Count 2 (Claim 1 of the '269 Patent)

Pergolizzi Claims 536-37 correspond substantially to Count 2. Side-by-side comparisons of Count 2 and Claims 536-37 appear below and in Appendix 4. Also appearing below and in Appendix 4 is Pergolizzi Claim 443, from which Claims 536-37 depend.

Count 2 (Claim 1 of the US4882269)	**Claims 443 and 536-37 of Pergolizzi Application**
A method for the detection of a target nucleotide sequence, comprising: (a) contacting the target nucleotide under conditions that permit hybridization with (i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and (ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and (b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes	443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising: providing a composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable

are bound to different portions of the primary probe tail.	of providing a detectable signal; forming a complex comprising said composition and said analyte; and detecting said analyte by a signal provided by said signal generating portion or portions present in said complex. 536. The process of claim 443, wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte. 537. The process of claim 536, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.

Discussed below are features shared by Count 2 and Pergolizzi Claims 536-37. Not discussed below are certain shared features whose explicit or inherent presence in Claims 536-37 is immediately apparent. Note also that some of the features discussed below may represent variants that are obvious under 35 U.S.C. 103(a), even if they are not identified as such herein.

Target Polynucleotide/Analyte. Pergolizzi Claim 536 explicitly identifies the analyte as a polynucleotide. Furthermore, in Pergolizzi Claim 443 the first portion of the bridging entity is capable of "hybridizing" to the analyte, clearly implying that the analyte can be a polynucleotide.

Polymeric Tail/Second Portion. In certain Pergolizzi claims, the second portion of the bridging entity corresponds to the "polymeric tail" of Count 2. The word "tail" means only that it does not bind to the target polynucleotide and that it is free to bind to the secondary probes.[22] Thus, in the

[22] The '269 Patent defines the tail as follows:

> The "primary probe" comprises a polynucleotide sequence which is complementary to the target sequence of interest attached to a "tail" that does not bind to the target sequence and is available for binding to other substances. The "tail" of the primary probe may comprise any of a number of polymers, including but not limited to single and double-stranded polynucleotides, and other natural or synthetic polymers such as cellulose, nylon, rayon, and the like. The primary probe may comprise linear or circular molecules. '269 Patent, col. 5, line 15.

Pergolizzi claims the second portion is equivalent to a tail. The precise meaning of "polymeric" as used in Count 2 is unclear, but it may mean that the tail includes a molecule, preferable a polynucleotide, that contains some amount of repeating residues.[23] Pergolizzi Claim 537 recites that the second portion comprises a low-complexity nucleic acid segment with repeating residues.

Incapable of Binding to Target Sequence/Incapable of Binding to Analyte. In Count 2, the segments of the tail cannot bind to the analyte. Pergolizzi Claim 536 similarly recites that the segments of the second portion cannot bind to the analyte.

Family of Signal-Generating Probes/Signalling Entities and Signal-Generating Portions. The Pergolizzi claims do not explicitly recite a "family" of signal-generating probes wherein each member of the family can bind to a "different" site on the tail of the primary probe. However, as used in the '269 Patent, "family" just means there is more than one signal-generating probe, and "different" binding sites just means there is more than one binding site—because a family of probes cannot bind if there is only a single binding site for only a single member of the family.[24] Similarly, Pergolizzi Claim 443 recites "a[25] second portion comprising one or more nucleic acid sequences or segments," "one or more signalling entities," "each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion," and "one or more signal generating portions." Thus, Claim 443 inherently recites a family of signal-generating probes each member of which can bind to a different site on the second portion of the bridging entity. Pergolizzi Claim 537 further indicates that many signalling entities bind to the second portion of the bridging entity.

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[23] *See* '269 Patent, col. 8, lines 28-46.

[24] *See, e.g.*, File history of the '269 Patent, pages 7-11 and 13-14 of the Amendment filed February 28, 1989 for Application No. 940,712.

[25] The article "a" encompasses the plural. *See* KCJ *Corp. v. Kinetic Concepts Inc.*, 55 USPQ2d 1835, 1838-39 (Fed. Cir. 2000) (Unless the claim is specific as to the number of elements, the article "a" receives a singular interpretation only in rare circumstances when the patentee evinces a clear intent to so limit the article.); *Crystal Semiconductor Corp. v. TriTech Microelectronics International Inc.*, 57 USPQ2d 1953 (Fed. Cir. 2001); *North American Vaccine Inc. v. American Cyanamid Co.*, 28 USPQ2d 1333, 1336 (Fed. Cir. 1993); *Tate Access Floors Inc. v. Maxcess Technologies Inc.*, 55 USPQ2d 1513, 1517 (Fed. Cir. 2000); *Elkay Manufacturing Co. v. Ebco Manufacturing Co.*, 52 USPQ2d 1109, 1111-12 (Fed. Cir. 1999).

Amplification/Amplification. Claim 537 explicitly recites amplification.[26]

Accordingly, Pergolizzi Claims 536-37 correspond substantially to Count 2.

C. Count 3 (Claim 25 of the '269 Patent)

Pergolizzi Claims 538-39 correspond substantially to Count 3. Side-by-side comparisons of Count 3 and Claims 538-39 appear in Appendix 4 and in the table below. Also appearing below and in Appendix 4 is Pergolizzi Claim 411, from which Claims 538-39 depend.

Count 3 (Claim 25 of the US4882269)	**Claims 411 and 538-39 of Pergolizzi Application**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.	411. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof: (i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and (ii) a container carrying more than one non-radioactive signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequence or segment, and one or more signal generating portions, each such portion being capable of providing a detectable signal. 538. The kit of claim 411, wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing

[26] Note that the amplification achieved in Count 2 is simply the amplification that occurs whenever more than one signal attaches (directly or indirectly) to a primary probe that is attached (directly or indirectly) to the analyte. *See* '269 Patent, col. 22, lines 56-58, col. 4, lines 65-67, col. 4, line 67 to col. 5, line 6, and col. 22, lines 56-58.

	to the analyte. 539. The kit of claim 538, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.

Pergolizzi Claims 538-39 correspond substantially to Count 3 for the same reasons that Pergolizzi Claims 536-37 correspond substantially to Count 2.

D. Count 4 (Claim 53 of the '246 Patent)

Count 4 is directed to "A nucleic acid hybridization assay...." It is unclear whether Count 4 encompasses a kit, a method or a composition. The word "assay" can be either a noun (which implies a kit or composition) or a verb (which implies a method). Further, Count 4 appears to recite steps (which implies a method) but the steps are in the past tense rather than the gerund form customarily used for method claims. Also, Count 4 is substantively identical to Claim 53 and Claim 53 is recited as if it is in independent form though it expressly refers to Claim 51 and incorporates both Claim 51 and Claim 39, which are composition claims.

In view of the ambiguous classification of Count 4, it is compared below and in Appendix 4 with Pergolizzi process Claims 540-41, composition Claims 542-43 and kit Claims 544-45. The independent claims from which Claims 540-45 depend are Also appearing below and in Appendix 4.

Count 4 (Claim 53 of the US5124246)	**Claims 443 and 540-41 of Pergolizzi Application**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled	443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising: providing a composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said

oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal; forming a complex comprising said composition and said analyte; and detecting said analyte by a signal provided by said signal generating portion or portions present in said complex. 540. The process of claim 443, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments. 541. The process of claim 540, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences, said bridging entity first portion is capable of encoding a gene product or fragment thereof, and the process further comprises one or more washing steps prior to detection.
Count 4 (Claim 53 of the US5124246)	**Claims 283 and 542-43 of Pergolizzi Application**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which	283. A composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more non-radioactive signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity

are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal. 542. The composition of claim 283, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments. 543. The composition of claim 542, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences and said bridging entity first portion is capable of encoding a gene product or fragment thereof.
Count 4 (Claim 53 of the US5124246)	**Claims 411 and 544-45 of Pergolizzi Application**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;	411. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof: (i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and (ii) a container carrying more than one non-radioactive signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequences or segments, and one or more signal generating portions, each such portion being capable of providing a detectable signal. 544. The kit of claim 411, wherein said analyte is a single-stranded DNA sequence fixed

II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments. 545. The kit of claim 544, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences and said bridging entity first portion is capable of encoding a gene product or fragment thereof.

Discussed below are features shared by Count 4 and Pergolizzi Claims 540-45. Not discussed below are certain shared features whose explicit or inherent presence in Claims 540-45 is immediately apparent. Note also that some of the features discussed below may represent variants that are obvious under 35 U.S.C. 103(a), even if they are not identified as such herein.

First Single-Stranded Nucleic Acid of Interest/Analyte. As indicated by the dependent claims of the '246 Patent (such as Claim 48), the first sequence of interest is simply the analyte or a nucleic acid that can bind to it. Thus, the first sequence of interest is substantially the same as the analyte in the Pergolizzi claims.

Second Single-Stranded Nucleic Acid of Interest/Signalling Entities. As indicated by the dependent claims of the '246 Patent (such as Claim 51), the second sequence of interest is simply a signal probe or a nucleic acid that can bind to it. Thus, the second sequence of interest is substantially the same as the signalling entities in the Pergolizzi claims.

Nucleic Acid Analyte/DNA Analyte. Pergolizzi Claims 540-45 explicitly recite that the analyte is a DNA sequence. Furthermore, in Pergolizzi Claims 283, 411 and 443, the first portion of the bridging entity is capable of "hybridizing" to the analyte, clearly implying that the analyte can be a polynucleotide.

Analyte Bound to a Solid Phase/Analyte Fixed to a Solid Support. Pergolizzi Claims 540-45 explicitly recite that the analyte is fixed to a solid support.

Single-Stranded Analyte/DNA Analyte. Claims 540-45 recite that the analyte is single-stranded. Furthermore, in the Pergolizzi claims single-strandedness is inherent because the DNA analyte must be single-stranded or must become single-stranded to hybridize to the bridging entity.

Multimer/Bridging Entity. The "nucleic acid multimer" of Count 4 is simply a multi-segment polynucleotide. More specifically, the multimer is a single-stranded polynucleotide with three or more segments, wherein one segment hybridizes to a first sequence of interest (analyte) and the other segments hybridize to a second sequence of interest (signal probe). As such, the multimer is substantially the same as Pergolizzi's bridging entity. In Pergolizzi Claims 540-45, the bridging entity comprises a multi-segment nucleic acid wherein one single-stranded polynucleotide segment (first portion) hybridizes to the analyte and at least two other single-stranded nucleic acid segments (second portion) hybridize to the signalling entities.

Single-Stranded Multimer/Bridging Entity. In Claims 540-545, the bridging entity is single-stranded because its two portions are single-stranded. Furthermore, single-strandedness is inherent in the Pergolizzi claims because the bridging entity must be single-stranded or must become single-stranded to hybridize to either the analyte or the signalling entities.

Synthetic Multimer/Bridging Entity. Count 4 specifies that the multimer is synthetic. Pergolizzi Claims 540-45 specify that the bridging entity is non-naturally occurring or artificially modified.

Linear Multimer/Bridging Entity. Count 4 specifies that the multimer is linear. Pergolizzi Claims 540-45 specify that both portions of the bridging entity are linear.

Nonhomopolymeric Multimer/Bridging Entity. As used in the '246 Patent, the term "nonhomopolymeric" essentially means a polynucleotide other than a low-complexity polynucleotide with repeating residues.[27] In other words, "nonhomopolymeric" means complex or

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[27] "Nonhomopolymeric" is not explained in the '246 Patent and appears nowhere in it except its claims. However, during prosecution the applicants for the '246 Patent stated that: "The term 'nonhomopolymeric' … is believed to be inherent in the [applicants'] use of the terms 'multimer' and 'oligonucleotide unit'." *See* File History of '246 Patent, Amendment of March 1, 1990 (p.9,11). Furthermore, at least one biotechnology dictionary defines the base word "homopolymer" as follows: "In general meaning, any polymeric molecule containing a single type of monomer. In molecular biology, the term refers to a short nucleic acid segment that consists of a single type of nucleotide, for example, oligo dT." Mark L. Steinberg, Ph.D. and Sharon D. Cosloy, Ph.D., The Facts on File Dictionary of Biotechnology and Genetic Engineering, p. 106 (2001). For the base word "homopolymer," this dictionary's definition appears to be the one that the '246 applicants had (continued…)

somewhat complex (as used in the '106 Patent). In Pergolizzi Claims 540-45, the bridging entity is nonhomopolymeric because: (1) it is a DNA sequence, (2) it must be nonhomopolymeric to bind to the nonhomopolymeric DNA sequence of the analyte, or (3) it encodes a gene product.

First Unit Covalently Bound to Second Unit/ Bridging Entity. In Count 4, the phrase "only via covalent bonds" essentially means that the segments of the multimer are not hybridized to each other. Rather, the segments have been ligated or cross-linked using conventional techniques.[28] Claims 540-45 recite that the first portion of the bridging entity is covalently bound to the second portion of the bridging entity. Furthermore, as read in light of the Pergolizzi specification, it is clear that other Pergolizzi claims inherently include this feature. In many or most of the Pergolizzi embodiments, the first portion of the bridging entity is in fact covalently bound to the second portion of the bridging entity.[29]

Unbound Labeled Oligonucleotide is Removed Prior to Detection/ Washing Step Prior to Detection. The step in Count 4 of removing unbound labeled oligonucleotide before detection of the signal is an obvious feature. In any event, method Claim 541 explicitly recites "one or more washing steps prior to detection."

Accordingly, Pergolizzi Claims 540-45 correspond substantially to Count 4.

(5) Identification of Support in Application for New Claims (37 C.F.R. § 1.607(a)(5))

Under 37 C.F.R. § 1.607(a)(5), Pergolizzi must show support for new claims designated as corresponding to the proposed counts. Since new Claims 532-45 are designated herein as corresponding to the counts, support for each feature recited in these claims appears below and in Appendix 5. Most of these features are explicitly recited in the Pergolizzi specification or original

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in mind. *See, e.g.*, File History of '246 Patent, Amendment of March 1, 1990 (p.11) and Amendment of February 19, 1991 (p.5-6).

[28] *See, e.g.*, '246 Patent, col. 9, lines 31-53 and col. 10, lines 28-36.

[29] *See, e.g.*, Pergolizzi Application, Figure 2, Page 22, beginning of last paragraph to Page 23 end of first paragraph.

Pergolizzi claims.[30] The few features that are not explicitly recited in the Pergolizzi specification or original claims are inherently or implicitly present in the specification or original claims.[31]

New Pergolizzi Process Claims 532-35	Support in Pergolizzi Application
532. The process of claim 443, wherein *said analyte is a DNA sequence, **said bridging entity is a single-stranded DNA sequence, and	*Original Claims 4, 7, 69, 102 and 147 (as filed May 5, 1983). Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2.

[30] Claims in an application constitute part of the original disclosure and may provide or contribute to compliance with 35 U.S.C. §112. ¶1. *See Ex Parte Wolters and Kuypers*, 214 USPQ 735, 736 (PTO Bd. App. 1982); *Ex parte Porter*, 25 USPQ2d 1144, 1146 (BPAI 1992); *Hyatt v. Boone*, 47 USPQ2d 1128, 1130 (Fed. Cir. 1998).

[31] *See generally Hall v. Taylor*, 332 F.2d 844, 141 USPQ 821, 824 (CCPA 1964) (The key to determining whether a disclosure supports a claim for interference purposes is whether the disclosure teaches the gist of the invention defined by the claim…[S]crutiny is [also] required to get at the essence of what the count puports to define.); *Ex parte Holt*, 19 USPQ2d 1211, 1213 (PBAI 1991) (It is well established that the invention claimed need not be described *ipsis verbis* in order to satisfy the disclosure requirement of §112.); *In re Smythe*, 480 F. 2d 1376, 178 USPQ 279 (CCPA 1973) (By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it.); *Behr v. Talbot*, 27 USPQ2d 1401, 1407 (BPAI 1992) (It is not necessary for the application to reveal a conscious appreciation on the part of the applicants of the significance of the limitation in question.); *Ex parte Parks*, 30 USPQ2d 1234, 1236 (BPAI 1994) (Clearly, the observation of a lack of literal support does not, in and of itself, establish a prima facie case for lack of adequate descriptive support….In the situation before us, it cannot be said that the originally-filed disclosure would not have conveyed to one having ordinary skill in the art that appellants had possession of the *concept* of conducting the decomposition step generating nitric acid in the absence of a catalyst.); *Ex Parte Yamaguchi*, 6 USPQ2d 1805, 1807 (PTO Bd App & Int 1987) (It is well settled in patent law that a compound and all of its properties are inseparable. Accordingly, where a compound is disclosed in such a manner as to comply with §112, 1st para., the later addition of symbols by which the compounds can be identified, classified and compared (such as x-ray diffraction spectra, graphic formula, chemical nomenclature) do not define a separate invention.); *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970) ("[W]here no explicit description of a generic invention is to be found in the specification mention of representative compounds may provide an implicit description upon which to base generic claim language."); *In re Alton*, 37 USPQ2d 1578, 1581 (Fed. Cir. 1996) (If the specification contains a description of the claimed invention, albeit not in *ipsis verbis*, then the examiner or Board, in order to meet the burden of proof, must provide reasons why one of ordinary skill in the art would not consider the description sufficient.).

***said signalling entities are single-stranded DNA sequences.	**Original Claims 7, 20, 29, 30, 69, 125 and 147. Pergolizzi Specification p.10, 2nd para.; p.17, 2nd para.; pp.57-58; Fig.2. ***Original Claims 43 and 134. Pergolizzi Specification pp.57-58; Fig.2.
533. The process of claim 532, wherein said bridging entity is derived from a filamentous phage.	Original Claims 31, 32, 70, 111, 126-27 and 148. Pergolizzi Specification p.16, 2nd para; pp.57-61.
534. The process of claim 533, wherein said signalling entities are derived from filamentous phages.	Original Claims 39, 44, 45, 67, 71, 135-36 and 149. Pergolizzi Specification p.16, 2nd para; pp.57-61.
535. The process of claim 534, wherein *said bridging entity codes for a gene product or fragment thereof, and **said forming step comprises either (i) contacting said analyte with said bridging entity to form a first complex and thereafter contacting said first complex with said signalling entities to form said detectable complex or (ii) contacting said bridging entity with said signalling entities to form a first complex and thereafter contacting said first complex with said analyte to form said detectable complex.	*Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.; p.17, 2nd para.; p.30, 2nd and 3rd paras. **Pergolizzi Specification p.28, 2nd para.; p.31 4th para. to p.34, 1st para.; Figs.1-2.
New Pergolizzi Process Claims 536-37	**Support in Pergolizzi Application**
536. The process of claim 443, wherein *said analyte is a polynucleotide, **said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and ***and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2. **Original Claims 18 and 113. Pergolizzi Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2. ***Pergolizzi Specification p.14, 2nd para; Fig.2.
537. The process of claim 536, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.	Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.

New Pergolizzi Kit Claims 538-39	**Support in Pergolizzi Application**
538. The kit of claim 411, wherein *said analyte is a polynucleotide, **said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and ***and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2. **Original Claims 18 and 113. Pergolizzi Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2. ***Pergolizzi Specification p.14, 2nd para; Fig.2.
539. The kit of claim 538, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.	Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.; p.22, 1st para.
New Pergolizzi Method Claims 540-41	**Support in Pergolizzi Application**
540. The process of claim 443, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support, **said bridging entity comprises non-naturally occurring or artificially modified DNA, ***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, ****said bridging entity first portion is covalently bound to said bridging entity second portion, and *****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para. **Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para. ***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para.. ****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2. *****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.
541. The process of claim 540, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences, **said bridging entity first portion is	*Original Claims 43 and 134. Pergolizzi Specification pp.57-58. **Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.

capable of encoding a gene product or fragment thereof, and ***the process further comprises one or more washing steps prior to detection.	***Pergolizzi Specification p.18, 1st para.; p.28, 2nd para.
New Pergolizzi Composition Claims 542-43	**Support in Pergolizzi Application**
542. The composition of claim 283, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support, **said bridging entity comprises non-naturally occurring or artificially modified DNA, ***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, ****said bridging entity first portion is covalently bound to said bridging entity second portion, and *****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para. **Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para. ***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para.. ****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2. *****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.
543. The composition of claim 542, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences and **said bridging entity first portion is capable of encoding a gene product or fragment thereof.	*Original Claims 43 and 134. Pergolizzi Specification pp.57-58. **Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.
New Pergolizzi Kit Claims 544-45	**Support in Pergolizzi Application**
544. The kit of claim 411, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support, **said bridging entity comprises non-naturally occurring or artificially modified DNA, ***said bridging entity first portion comprises a linear single-stranded polynucleotide	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para. **Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para. ***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para..

sequence, ****said bridging entity first portion is covalently bound to said bridging entity second portion, and *****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.	****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2. *****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.
545. The kit of claim 544, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences and **said bridging entity first portion is capable of encoding a gene product or fragment thereof.	*Original Claims 43 and 134. Pergolizzi Specification pp.57-58. **Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.

(6) How Requirements of 35 U.S.C. § 135(b) Were Met (37 C.F.R. § 1.607(a)(6))

Section 135(b) requires that there was once pending,[32] on or before the one-year anniversary of the issuance of a patent in question, at least one application claim directed to the "same or substantially the same subject matter" as at least one claim in the patent.[33] The application claim need not define an identical invention.[34] Indeed, the claims need not even overlap.[35] But the

[32] Claims can satisfy Section 135(b) even if they were canceled prior to issuance of the patent in question. *See Tezuka v. Wilson*, 224 USPQ 1030 (PTO Bd. Pat. Int. 1984). *See also In re Berger*, 61 USPQ2d 1523, 1527-1528 (CAFC 2002); *Corbett v. Chisholm*, 568 F.2d 759, 765-66, 196 USPQ 337, 342 (CCPA 1977); *In re Schutte*, 244 F.2d 323, 326, 113 USPQ 537, 540 (CCPA 1957).

[33] *See also* 37 C.F.R. § 1.607(a)(6) and 37 C.F.R. § 1.601(j).

[34] *See Case v. CPC Int'l Inc.*, 730 F.2d 745, 749, 221 USPQ 196, 200 (Fed. Cir 1984), *cert. denied*, 469 US 872, 224 USPQ 736 (1984) (regarding interference-in-fact).

[35] *See Melony v. Arni*, 547 F.2d 566, 570, 192 USPQ 486, 489-90 (CCPA 1977) (regarding interference-in-fact).

application claim should explicitly or inherently contain or result in the material limitations recited in at least one of the patent claims.[36]

The one-year anniversary dates of the four interfering patents are: December 29, 1988 for the '106 Patent; November 21, 1990 for the '269 Patent; June 23, 1993 for the '188 Patent; and June 13, 1996 for the '246 Patent. Attached Appendix 6 sets forth a small selection of the many Pergolizzi claims that were pending before the one-year anniversary dates of the three earlier-issued patents, *i.e.*, before December 29, 1988, before November 21, 1990, or before June 23, 1993. The selected Pergolizzi claims do not explicitly recite each and every last one of the phrases explicitly recited in all four of the proposed counts. For the reasons discussed below, however, the selected Pergolizzi claims inherently contain or necessarily result in the limitations represented by these phrases.

A. Claims 1-10 of the '106 Patent

Below and in attached Appendix 6 is a comparison of Claim 1 of the '106 Patent (Count 1) with (1) Pergolizzi Claims 69-71, which appeared in the original Pergolizzi parent application (491,929) filed May 5, 1983, and (2) Pergolizzi Claims 155, 156, 159, 161, 165 and 168, which appeared in Pergolizzi's Amendment of June 3, 1985. Also appearing below and in Appendix 6 are the claims from which these Pergolizzi claims depend.

Claim 1 of US4716106 (Count 1)	**Claims 1 and 69-71 of Original Pergolizzi Application**
A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of (a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and (b) a polynucleotide primary probe	1. A method of detecting in a sample an analyte (A) having a molecularly recognizable portion thereon, which comprises: providing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable

[36] *See, e.g., In re Berger*, 61 USPQ2d 1523, 1527-1528 (CAFC 2002) (A material limitation need not be expressed explicitly, but may be expressed inherently if the material limitation necessarily results from other limitations in the claim.); *Berman v. Housey*, 63 USPQ2d 1023, 1030-31 (Fed. Cir. 2002); *Corbett v. Chisholm*, 568 F.2d 759, 765-66, 196 USPQ 337, 343 (CCPA 1977); *In re Schutte*, 244 F.2d 323, 326, 113 USPQ 537, 540 (CCPA 1957). *See also Parks v. Fine*, 773 F.2d 1577, 1579, 227 USPQ 432, 434 (CAFC 1985); *Connin v. Andrews*, 223 USPQ 243, 247 (PTO Bd. Pat. Intf. 1984).

having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of (i) contacting the sample under hybridisation conditions with the primary probe, (ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and (iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.	portion on said analyte; and (ii) a portion comprising a polynucleotide sequence; and (C) a signalling entity having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity, thereby to form a stable polynucleotide hybrid, and (ii) a signal generating portion; forming a complex comprising: (1) said analyte (A) complexed through said molecularly recognizable portion to (2) said recognizing portion of said entity (B); said entity (B) being complexed through said polynucleotide portion thereon to (3) said polynucleotide portion of said signalling entity (C); and detecting a signal by means of said signal generating portion present in said complex. 69. The method of Claim 1 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said step of detection by means of said signal generating portion on said signalling entity is based on non-radioactive detection. 70. The method of Claim 69 wherein said bridging entity is derived from a filamentous phage. 71. The method of Claim 69 wherein said signalling entity is derived from a filamentous phage.
Claim 1 of US4716106 (Count 1)	**Claims 154-56, 159, 161, 165 and 168 of Pergolizzi's Amendment of June 3, 1985**
A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of (a) a labelled polynucleotide secondary probe having a complex single-stranded	154. A method of detecting in a sample an analyte having a molecularly recognizable portion thereon, comprising: forming a detectable complex comprising

polynucleotide sequence, and (b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of (i) contacting the sample under hybridisation conditions with the primary probe, (ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and (iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.	(a) said analyte, (b) a non-naturally-occurring molecular bridging entity comprising a portion capable of recognizing and complexing to said molecularly recognizable analyte portion, and a portion comprising a polynucleotide sequence, and (c) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion and a signal generating portion; and detecting said analyte by a signal provided by said signal generating portion present in said detectable complex. 155. The method according to claim 154, characterized in that said forming step comprises contacting said analyte with said bridging entity to form a first complex and contacting said first complex with said signalling entity to form said detectable complex. 156. The method according to claim 154, characterized in that said forming step comprises contacting said bridging entity with said signalling entity to form a first complex and contacting said first complex with said analyte to form said detectable complex. 159. The method according to claim 158, characterized in that said molecularly recognizable portion on said analyte is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence, and a protein. 161. The method according to claim 154, characterized in that said bridging entity recognizing portion is an RNA or DNA oligo- or polynucleotide sequence. 165. The method according to claim 154, characterized in that said bridging entity is selected from the group consisting of a single-stranded, double stranded or partially double-stranded circular DNA polymer, a circular DNA polymer derived from a filamentous phage, an M13 phage or a variant thereof. 168. The method according to claim 154, characterized in that said signalling entity is selected from the group consisting of a single stranded, double stranded, or partially double-

	stranded polynucleotide polymer, a naturally occurring modified DNA, a polynucleotide polymer derived from a T (even) phage, a modified DNA carrying a cloned insert, a polymer derived from a filamentous phage, M13 phage or a variant thereof, and a polymer derived from a circular DNA molecule covalently attached to a non radiolabelled signal generating portion.

Discussed below are select limitations shared by Claim 1 of the '106 Patent and the Pergolizzi claims in the table above. Not discussed below are certain shared limitations whose explicit or inherent presence in the Pergolizzi claims is immediately apparent. Note also that some of the limitations discussed below may be immaterial even if they are not identified as such herein.

Specific Target Polynucleotide/Analyte. Pergolizzi Claims 69-71 and 159 recite that the recognizable portion on the analyte is a polynucleotide. When the recognizable portion on the analyte is a polynucleotide, at least the first portion of bridging entity is a polynucleotide specific for particular polynucleotide analytes.

Single-Stranded Primary Probe/Bridging Entity. Claims 69-71 and 165 recite that the bridging entity is single-stranded. Furthermore, single-strandedness is inherent because the bridging entity must be single-stranded or must become single-stranded to hybridize to either the analyte or the signalling entities.[37]

Single-Stranded Secondary Probe/Signalling Entities. Claim 168 recites that the signalling entities may be single-stranded. Claim 71 recites that the signalling entities are derived from filamentous phages, which are single-stranded. Furthermore, single-strandedness is inherent because the signalling entities must be single-stranded or must become single-stranded to hybridize to the bridging entity.

Complex Primary Probe/Bridging Entity. As used in the '106 Patent, "complex" means only that the polynucleotide is not a low-complexity sequence with uniform or highly repetitive residues.[38] In Claims 69-71, 159, 161, 165 and 168, the bridging entity is complex because: (1) it must be complex

[37] *See, e.g.*, '106 Patent, col. 3, lines 3-11.

[38] *See, e.g.*, '106 Patent, col. 2, lines 39-43.

to bind to the complex sequences of the analyte or signalling entities and/or (2) it is a DNA polymer derived from a filamentous phage such as M13.[39]

Complex Secondary Probe/Signalling Entities. In Claims 69-71, 165 and 168, the signalling entities are or can be complex because: (1) they must be complex to bind to a complex second portion of the bridging entity and/or (2) because they are DNA polymers derived from filamentous phages such as M13.[40]

Before, During or After/Variable Order of Forming Complex. Claims 155 and 156 recite that either (1) the analyte and bridging entity can first form a complex that in turn forms a second complex with the signalling entities or (2) the signalling entities and bridging entity can first form a complex that in turn forms a second complex with the analyte.

Accordingly, Pergolizzi Claims 69-71, 154-56, 159, 161, 165 and 168 recite or necessarily result in the material limitations recited in Claim 1 of the '106 Patent. Because the features added by dependent Claims 2-10 of the '106 Patent are immaterial, the Pergolizzi claims are also directed to substantially the same subject matter as Claims 2-10 of the '106 Patent.

B. Claims 1-24 of the '269 Patent

Below and in attached Appendix 6 is a comparison of Claim 1 of the '269 Patent (Count 2) with Pergolizzi Claims 159 and 161-65, which first appeared in Pergolizzi's Amendment of June 3, 1985. Also appearing below and in Appendix 6 is Pergolizzi Claim 154, from which Pergolizzi Claims 159 and 161-65 depend.

Claim 1 of US4882269 (Count 2)	**Claims 154 and 161-65 of Pergolizzi's Amendment of June 3, 1985**
A method for the detection of a target nucleotide sequence, comprising: (a) contacting the target nucleotide under	154. A method of detecting in a sample an analyte having a molecularly recognizable portion thereon, comprising:

[39] *See also* Claim 16 of the Original Pergolizzi Application: "16. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity codes for a gene product or fragment thereof."

[40] *See also* Claim 34 of the Original Pergolizzi Application: "34. The method of Claim 1 wherein said polynucleotide portion on said signalling entity codes for a gene product or fragment thereof."

conditions that permit hybridization with (i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and (ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and (b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.	forming a detectable complex comprising (a) said analyte, (b) a non-naturally-occurring molecular bridging entity comprising a portion capable of recognizing and complexing to said molecularly recognizable analyte portion, and a portion comprising a polynucleotide sequence, and (c) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion and a signal generating portion; and detecting said analyte by a signal provided by said signal generating portion present in said detectable complex. 159. The method according to claim 158, characterized in that said molecularly recognizable portion on said analyte is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence, and a protein. 161. The method according to claim 154, characterized in that said bridging entity recognizing portion is an RNA or DNA oligo- or polynucleotide sequence. 162. The method according to claim 154, characterized in that said bridging entity polynucleotide portion is a polynucleotide sequence of low complexity. 163. The method according to claim 162, characterized in that said bridging entity polynucleotide sequence is selected from the group consisting of a polydeoxy G, polydeoxy C, polydeoxy T or polydeoxy A. 164. The method according to claim 161, characterized in that said bridging entity polynucleotide portion and said bridging entity recognizing portion are not capable of hybridizing to identical oligo- and polynucleotide sequences. 165. The method according to claim 154, characterized in that said bridging entity is selected from the group consisting of a single-stranded, double stranded or partially double-stranded circular DNA polymer, a circular DNA polymer derived from a filamentous phage, an M13 phage or a variant thereof.

Discussed below are select limitations shared by Claim 1 of the '269 Patent and Pergolizzi Claims 159 and 161-65. Not discussed below are certain shared limitations whose explicit or inherent presence in Claims 159 and 161-65 is immediately apparent. Note also that some of the limitations discussed below may be immaterial even if they are not identified as such herein.

Target Polynucleotide/Analyte. Claims 159, 161 and 164-65 indicate that the analyte is a polynucleotide. Furthermore, as described throughout the Pergolizzi Application, the chief use for the invention is to detect polynucleotides.

Polymeric Tail/Second Portion. In Claims 159 and 161-65, the second portion of the bridging entity is equivalent to the "polymeric tail" of Claim 1 of the '269 Patent.[41] The word "tail" means only that it does not bind to the target polynucleotide and that it is free to bind to the secondary probes.[42] Thus, in the Pergolizzi claims the second portion is equivalent to a tail. The precise meaning of "polymeric" as used in Claim 1 of the '269 Patent is unclear, but it may mean that the tail includes a molecule, preferable a polynucleotide, that contains some amount of repeating residues. Claims 162-63 explicitly recite that the second portion is a low-complexity polynucleotide.

Incapable of Binding to Target Sequence/Incapable of Binding to Analyte. In Claim 164, the bridging entity first and second portion are substantially incapable of binding to the same polynucleotide (*i.e.*, the analyte). Thus, the signalling entities must also be substantially incapable of binding to the analyte.[43]

Family of Signal-Generating Probes/Signalling Entities and Signal-Generating Portions. Claims 159 and 161-65 do not explicitly recite a "family" of signal-generating probes wherein each member of the family can bind to a "different" site on the tail of the primary probe. However, as used in the '269 Patent, "family" just means there is more than one signal-generating probe, and "different" binding sites just means there is more than one binding site—because a family of probes cannot bind if there

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[41] *See* '269 Patent, col. 5, line 15.

[42] *See* '269 Patent, col. 8, lines 38-46.

[43] Since the signalling entities are complementary to the second portion, the signalling entities could bind to the analyte only if the analyte were identical or highly homologous to the second portion. As evident from the Pergolizzi Application, the second portion will rarely if ever be identical or highly homologous to the analyte. *See, e.g.*, Pergolizzi Specification, p.15, first para.

is only a single binding site for only one member of the family.[44] Likewise, the Pergolizzi claims tacitly recite more than one signalling entity. For example, Claim 154 recites (1) "a" second portion comprising (2) "a" polynucleotide sequence and (3) "a" signalling entity comprising (4) "a" polynucleotide portion capable of annealing to the second portion and (5) "a" signal generating portion.[45] In patent claims, the article "a" includes the plural. Thus, Claims 159 and 161-65 inherently include a family of signal-generating probes each member of which can bind to a different site on the second portion of the bridging entity.

Amplified Signal/Amplification. Claims 159 and 161-65 do not explicitly recite amplification of signals, but, as shown in the above paragraph, they inherently include it. The amplification achieved in Claim 1 of the '269 Patent is simply the amplification that occurs whenever more than one signal attaches directly or indirectly to a primary probe that is attached directly or indirectly to the analyte.[46]

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[44] *See, e.g.*, file history of the '269 Patent, pages 7-11 and 13-14 of the Amendment filed February 28, 1989 for Application No. 940,712.

[45] Some Pergolizzi claims pending before November 21, 1990 explicitly recite "more than one" signalling entity. *See, e.g.*, Claims 184-186, 190-192 and 196 of Pergolizzi's Amendment of October 9, 1990 or October 26, 1990. Below is Claim 196 of Pergolizzi's Amendment of October 9, 1990:

> 196. A method of detecting in a sample an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising:
>
> forming a detectable complex comprising (a) said analyte, (b) a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable analyte portion, and a **second portion comprising more than one polynucleotide sequence,** and (c) **more than one signalling entity**, each said signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with the polynucleotide sequences in said bridging entity second portion and a signal generating portion; and
>
> detecting said analyte by an **amplified signal** provided by said signal generating portions present in said detectable complex, wherein at least one component of said detectable complex is immobilized or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portions thereof.

[46] *See* '269 Patent, col. 22, lines 56-58, col. 4, lines 65-67, col. 4, line 67 to col. 5, line 6, col. 22, lines 56-58.

Furthermore, as evident in the Pergolizzi Application, this type of amplification—many signals per detection probe—was well-known in the art.[47]

Accordingly, Claims 159 and 161-65 recite or necessarily result in the material limitations recited in Claim 1 of the '269 Patent. Because the limitations added by dependent Claims 2-24 of the '269 Patent are immaterial, Claims 159 and 161-65 are also directed to substantially the same subject matter as Claims 2-24 of the '269 Patent.

C. Claims 25-48 and 49-62 of the '269 Patent and Claims 1-19 of the '188 Patent

(i) Claims 25-48 of '269 Patent:

Below and in attached Appendix 6 is a comparison of kit Claim 25 of the '269 Patent (Count 3) with kit Claims 102 and 147-48 of the original Pergolizzi application filed in 1983. Also appearing below and in Appendix 6 is Claim 100, from which Claims 102 and 147-48 depend.

Claim 25 of US4882269 (Count 3)	**Claims 100, 102 and 147-48 of Original Pergolizzi Application**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to	100. A kit useful for the detection of an analyte (A) having a molecularly recognizable portion thereon, comprising; I) a carrier being compartmentalized to receive in close confinement therein one or more container means; II) a first container means containing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable portion on said analyte (A); and (ii) a portion comprising a polynucleotide sequence; and (III) a second container means containing a signalling entity (C) having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide

[47] *See* Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para. Furthermore, other Pergolizzi claims pending before November 21, 1990 explicitly recite amplification via attachment of more than one signal. *See, e.g.*, Claim 196 immediately above.

different portions of the primary probe tail.	portion of said bridging entity (B) thereby by form a stable polynucleotide hybrid; and (ii) a signal generating portion. 102. The kit of Claim 100 wherein said recognizing portion on said bridging entity comprises a polynucleotide sequence. 147. The kit of Claim 100 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said signal generating portion on said signalling entity is based on non-radioactive detection. 148. The kit of Claim 147 wherein said bridging entity is derived from a filamentous phage.

Pergolizzi kit Claims 102 and 147-48 are directed to substantially the same invention as Claims 25-48 of the '269 Patent (Count 3) for the same reasons (see above) that Pergolizzi method Claims 159 and 161-65 are directed to substantially the same invention as Claims 1-24 of the '269 Patent (Count 2).

(ii) Claims 49-62 of '269 Patent:

The reasons above also explain why Pergolizzi kit Claims 102 and 147-48 are directed to substantially the same subject matter as Claims 49-62 of the '269 Patent. Independent Claim 49 of the '269 Patent differs from Count 3 only in that Claim 49 recites "a primary probe cassette which comprises a cloning vector" and "a multiple cloning site into which a target nucleotide sequence can be inserted and cloned." No Pergolizzi claims contain these phrases. However, the bridging entities of the Pergolizzi claims encompass various types of polynucleotide bridging entities including the cassette and vector type polynucleotides described in the '269 Patent.[48] Furthermore,

[48] Cassette and vector type polynucleotides are the immediate precursors of detection probes and have long been used to clone detection probes for use in assays. *See* '269 Patent, col. 8, lines 17-26, col. 2, lines 57-63, col. 5, lines 52-57, col. 8, lines 43-57.

in many of the Pergolizzi claims (such as Claim 148), the bridging entities are explicitly derived from phages.[49]

Because the limitations added by dependent Claims 50-62 of the '269 Patent are immaterial, Claims 102 and 147-48 are also directed to substantially the same subject matter as Claims 50-62.[50]

(iii) Claims 1-19 of '188 Patent:

For the same reasons that Pergolizzi kit Claims 102 and 147-48 are directed to substantially the same subject matter as Claims 25-62 of the '269 Patent, Pergolizzi kit Claims 102 and 147-48 are also directed to substantially the same subject matter as Claims 1-19 of the '188 Patent. The fact that the claims of the '188 Patent were terminally disclaimed over the claims of the '269 Patent underscores this point.

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[49] *See, e.g.*, Claims 126-128, 135-16 and 148 in original application and Claim 177 in Pergolizzi's Amendment of June 3, 1985, of January 7, 1986 or of June 29, 1989.

[50] Additional Pergolizzi claims are may also be directed to substantially the same subject matter as Claims 25-62 of the '269 Patent. *See, e.g.*, Claims 173, 175, 177 and 179 of Pergolizzi's Amendment of June 3, 1985; Claims 177 and 179 of Pergolizzi's Amendment of January 7, 1986; Claims 179 and 183 of Pergolizzi's Amendment of June 29, 1989; Claims 187-189 and 193 of Pergolizzi's Amendment of October 9, 1990; and Claims 187-189, 193 and 197 of Pergolizzi's Amendment of October 26, 1990. Below is Claim 197 of Pergolizzi's Amendment of October 26, 1990:

> 197. A kit for the detection in a sample of an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising as components thereof:
>
> (i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable portion on said analyte; and **a second portion comprising more than one polynucleotide sequence**; and
>
> (ii) a container carrying **more than one signalling entity**, each said signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with the polynucleotide sequences in said bridging entity second portion, and a signal generating portion;
>
> which molecular bridging entity and signalling entities form a detectable complex with said analyte, wherein at least one component of said detectable complex is immobilized or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portion thereof.

D. Claims 39-41, 43-52 and 53-56 of the '246 Patent:

Claim 53 of the '246 Patent (Count 4) is directed to "A nucleic acid hybridization assay." As explained earlier, it is unclear whether Claim 53 should be classified as a kit, a method or a composition. In view of the ambiguous classification of Claim 53, it is compared below and in Appendix 6 with various Pergolizzi kit, method and composition claims that were pending before June 23, 1993, the one-year anniversary date of the '246 Patent.

Claim 53 of US5124246 (Count 4)	**Claims 1, 4, 7, 16, 20 and 69 of Original Pergolizzi Application**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and	1. A method of detecting in a sample an analyte (A) having a molecularly recognizable portion thereon, which comprises: providing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable portion on said analyte; and (ii) a portion comprising a polynucleotide sequence; and (C) a signalling entity having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity, thereby to form a stable polynucleotide hybrid, and (ii) a signal generating portion; forming a complex comprising: (1) said analyte (A) complexed through said molecularly recognizable portion to (2) said recognizing portion of said entity (B); said entity (B) being complexed through said polynucleotide portion thereon to (3) said polynucleotide portion of said signalling entity (C); and detecting a signal by means of said signal generating portion present in said complex. 4. The method of Claim 1 wherein the **molecularly recognizable portion on said analyte comprises nucleic acid**. 7. The method of Claim 1 wherein said **recognizing portion on said bridging entity comprises a polynucleotide sequence**.

VI. the presence of label bound to the multimer is detected.	16. The method of Claim 1 wherein said **polynucleotide sequence on said bridging entity codes for a gene product or fragment thereof.** 20. The method of Claim 1 wherein said **polynucleotide sequence in said bridging entity is covalently attached to another polynucleotide sequence.** 69. The method of Claim 1 wherein said **recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence** capable of stably annealing thereto**, said bridging entity is a single-stranded DNA polymer**, and said step of detection by means of said signal generating portion on said signalling entity is based on non-radioactive detection.
Claim 53 of US5124246 (Count 4)	**Claims 172, 173 and 179 of Pergolizzi's Amendment of June 3, 1985**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the	172. A kit useful for the detection in a sample of an analyte having a molecularly recognizable portion thereon, comprising as components thereof: (i) **a non-naturally occurring molecular bridging entity** comprising a portion capable of recognizing said molecularly recognizable portion on said analyte; and a portion comprising a polynucleotide sequence; and (ii) a signalling entity comprising a polynucleotide portion capable of annealling to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion, and a signal generating portion. 173. The kit according to claim 172, characterized in that said **bridging entity recognizing portion is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence**, an antigen, an antibody, a saccharide, a lectin, a hormone, a receptor, an enzyme inhibitor, a cofactor bonding site, an enzyme active site, and a receptor protein. 179. The kit according to claim 172,

analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	characterized in that said **signalling entity is selected from the group consisting of a single-stranded**, double-stranded or partially double-stranded polynucleotide polymer, a naturally-occurring modified DNA, a polynucleotide polymer derived from a T (even) phage, a modified DNA carrying a cloned insert, a polymer derived from a filamentous phage, M13 phage or a variant thereof, and a polymer derived from a circular DNA molecule covalently attached to a non-radiolabelled signal generating portion.
Count 4 (Claim 53 of US5124246)	**Claim 193 of Pergolizzi's Amendment of Oct. 9, 1990**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is	193. A kit for the detection in a sample of an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising as components thereof: (i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable portion on said analyte; and as second portion comprising more than one polynucleotide sequence; and (ii) a container carrying a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity second polynucleotide portion, and a signal generating portion; which molecular bridging entity and signalling entity form a detectable complex with said analyte, wherein the **at least one component of said detectable complex is immobilized** or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portion thereof.

removed; and VI. the presence of label bound to the multimer is detected.	
Claim 53 of US5124246 (Count 4)	**Claim 196 of Pergolizzi's Amendment of Oct. 9, 1990**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	196. A method of detecting in a sample an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising: forming a detectable complex comprising (a) said analyte, (b) a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable analyte portion, and a second portion comprising more than one polynucleotide sequence, and (c) more than one signalling entity, each said signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with the polynucleotide sequences in said bridging entity second portion and a signal generating portion; and detecting said analyte by an amplified signal provided by said signal generating portions present in said detectable complex, wherein **at least one component of said detectable complex is immobilized** or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portions thereof.
Claim 53 of US5124246 (Count 4)	**Claims 194, 199, 209-213, 217-18 and 220 of Pergolizzi's Amendment of Dec. 22, 1992**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear	194. A composition of matter comprising: a molecular bridging entity comprising a

nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	first portion capable of recognizing and binding to a molecularly recognizable portion on an analyte, and a second portion comprising a nucleic acid; and a universal signalling entity comprising a nucleic acid portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity nucleic acid second portion, and a signal generating portion capable of providing, directly or indirectly, a detectable signal. 199. The composition according to claim 194, wherein said **analyte is selected from the group consisting of a nucleic acid** and a protein. 209. The composition according to claim 194, wherein said **molecular bridging recognizing first portion comprises a nucleic acid**. 210. The composition according to claim 209, wherein said **nucleic acid comprises an oligo- or polynucleotide**. 211. The composition according to claim 210, wherein **said oligo- or polynucleotide comprises a modified oligo- or polynucleotide**. 212. The composition according to claim 210, wherein said **oligo- or polynucleotide is single-stranded or partially double-stranded**. 213. The composition according to claim 210, wherein said **oligo- or polynucleotide is circular or linear**. 217. The composition according to claim 194, wherein said **polynucleotide sequence in the molecular bridging entity second portion is linear or circular**. 218. The composition according to claim 194, wherein said **nucleic acid in the molecular bridging entity second portion is single-stranded or partially double-stranded**. 220. The composition according to claim 194, wherein said **polynucleotide sequence in the molecular bridging entity second portion is derived from a phage selected from the group consisting of a T even phage, a filamentous phage, an M13 phage, or a variant thereof**.

Discussed below are select limitations shared by Claim 53 of the '246 Patent and the Pergolizzi claims in the table above. Not discussed below are certain shared limitations whose explicit or inherent presence in the Pergolizzi claims is immediately apparent. Note also that some of the limitations discussed below may be immaterial even if they are not identified as such herein.

First Single-Stranded Nucleic Acid of Interest/Analyte. The first sequence of interest is simply the analyte or a nucleic acid that can bind to it. Thus, the first sequence of interest is substantially the same as the analyte in the Pergolizzi claims.

Second Single-Stranded Nucleic Acid of Interest/Signalling Entities. The second sequence of interest is simply a signal probe or a nucleic acid that can bind to it. Thus, the second sequence of interest is substantially the same as the signalling entities in the Pergolizzi claims.

Nucleic Acid Analyte/Analyte. Pergolizzi Claims 4, 69 and 199 recite (and Pergolizzi Claims 7, 172, 173 and 209 imply) that the analyte is a DNA or nucleic acid sequence.

Analyte Bound to a Solid Phase/Analyte. Pergolizzi Claims 193 and 196 indicate that the analyte can be immobilized.

Single-Stranded Analyte/Analyte. In the Pergolizzi claims, single-strandedness is inherent because the DNA analyte must be single-stranded or must become single-stranded to hybridize to the DNA bridging entity.

Multimer/Bridging Entity. As explained earlier, the "nucleic acid multimer" of Claim 53 of the '246 Patent is substantially the same as Pergolizzi's bridging entity.

Single-Stranded Multimer/Bridging Entity. Claims 69, 212 and 218 explicitly recite that the bridging entity is or may be single-stranded. Furthermore, single-strandedness is inherent in the Pergolizzi claims because either portion of the bridging entity must be single-stranded or must become single-stranded to hybridize to the analyte or signal probes.

Synthetic Multimer/Bridging Entity. Pergolizzi Claims 172 and 211 specify that the bridging entity is non-naturally occurring or artificially modified.

Linear Multimer/Bridging Entity. Pergolizzi Claims 213 and 217 specify that the first and second portions of the bridging entity are linear.

Nonhomopolymeric Multimer/ Bridging Entity. As used in the '246 Patent, the term "nonhomopolymeric" essentially means a polynucleotide other than a low-complexity polynucleotide with repeating residues. [51] In Pergolizzi Claims 4, 7, 16, 69, 172, 173, 179, 199, 209 and 220, the bridging entity is nonhomopolymeric because: (1) it is a DNA sequence, (2) it must be nonhomopolymeric to bind to the nonhomopolymeric DNA sequence of the analyte, (3) it must be nonhomopolymeric to bind to the nonhomopolymeric DNA of the signalling entity, (4) it is derived from a filamentous phage, or (4) it encodes a gene product.

First Unit Covalently Bound to Second Unit/ Bridging Entity. Pergolizzi Claim 20 explicitly recites that the first portion of the bridging entity is covalently bound to another polynucleotide. Furthermore, although most other Pergolizzi claims pending before June 23, 1993 do not recite that the first and second portions of the bridging entity are covalently bound, as read in light of the Pergolizzi specification, it is clear that these claims inherently include this limitation. In other words, as envisioned by Pergolizzi, the first and second portions of the bridging entity are covalently bound to each other.

Unbound Labeled Oligonucleotide is Removed Prior to Detection. The step in Claim 53 of removing unbound labeled oligonucleotide is immaterial. Such washing steps were the norm in this art.

Accordingly, the Pergolizzi claims in the table above are directed to substantially the same subject matter as Claim 53. Since Claim 53 incorporates Claims 39 and 51, and since Claims 40-41, 43-50, 52 and 54-56 add immaterial limitations, the Pergolizzi claims in the table above are also directed to substantially the same subject matter as Claims 39-41, 43-52 and 54-56.

CONCLUSION

Pergolizzi respectfully requests that an interference employing Proposed Count 1 be promptly declared between:

(I)(A) new Pergolizzi Application Claims 532-35, and

(II)(A) Claims 1-10 of U.S. Patent No. 4,716,106.

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[51] *See* File History of '246 Patent, Amendment of March 1, 1990 (p.9,11) and Amendment of February 19, 1991 (p.5-6).

Pergolizzi respectfully requests that an interference employing Proposed Count 2 be promptly declared between:

(I)(B) new Pergolizzi Application Claims 536-37, and

(II)(B) Claims 1-24 of U.S. Patent No. 4,882,269.

Pergolizzi respectfully requests that an interference employing Proposed Count 3 be promptly declared between:

(I)(C) new Pergolizzi Application Claims 538-39, and

(II)(C) Claims 25-69 of U.S. Patent No. 4,882,269 and Claims 1-19 of U.S. Patent No. 5,424,188.

Pergolizzi respectfully requests that an interference employing Proposed Count 4 be promptly declared between:

(I)(D) new Pergolizzi Application Claims 540-45, and

(II)(D) Claims 39-41 and 43-56 of U.S. Patent No. 5,124,246.

If the Examiner has any questions about this Request or the above-identified Application, the Examiner is invited to contact the undersigned attorneys.

Respectfully Submitted

HUNTON & WILLIAMS

Dated: March 28, 2003 By: _____

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APPENDIX 1

PATENTS THAT INTERFERE WITH APPLICATION

A. U.S. Patent No. 4,716,106

The '106 Patent issued on December 29, 1987, to David J. Chiswell for "Detecting Polynucleotide Sequences." It issued from Application No. 706,747, filed March 28, 1985, which claims the benefit of a British application filed March 1, 1984. Amersham International is the assignee named on the face of the '106 Patent. Pergolizzi first informed the Examiner that the '106 Patent interferes with the Pergolizzi Application in Pergolizzi's Amendment of July 25, 1997.

B. U.S. Patent No. 4,882,269

The '269 Patent issued on November 21, 1989, to Robert J. Schneider and Thomas E. Shenk for an "Amplified Hybridization Assay." It issued from Application No. 06/940,712, filed December 11, 1986, which is a continuation-in-part of Application No. 06/808,695, filed December 13, 1985. Princeton University is the assignee named on the face of the '269 Patent. Pergolizzi first informed the Examiner that the '269 Patent interferes with the Pergolizzi Application in Pergolizzi's Amendment of July 25, 1997.

C. U.S. Patent No. 5,424,188

The '188 Patent issued on June 13, 1995, to Robert J. Schneider and Thomas E. Shenk for an "Amplified Hybridization Assay." It issued from Application No. 07/963,923, filed October 20, 1992, which is a continuation of Application No. 07/400,831, filed August 29, 1989, which is a divisional of Application No. 06/940,712 (the '269 Patent), filed December 11, 1986, which is a

continuation-in-part of Application No. 06/808,695, filed December 13, 1985. Princeton University is the assignee named on the face of the '188 Patent. The '188 Patent is terminally disclaimed over the '269 Patent.

D. US Patent No. 5,124,246

The '246 Patent issued on June 23, 1992, to Michael S. Urdea, Brian Warner, and Thomas Horn for "Nucleic Acid Multimers and Amplified Nucleic Acid Hybridization Assays Using Same." The '246 Patent issued from Application No. 340,031, filed April 18, 1989, which is a continuation-in-part of Application No. 252,638, filed September 30, 1988, which is a continuation-in-part of Application No. 185,201, filed April 22, 1988, which is a continuation-in-part of Application No. 109,282, filed October 15, 1987. Chiron Corporation is the assignee named on the face of the '246 Patent. Pergolizzi first apprised the Examiner of the existence of the '246 Patent in Pergolizzi's Amendment of March 5, 1996.

APPENDIX 2

PROPOSED COUNTS

Proposed Count 1

A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of

(a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and

(b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of

(i) contacting the sample under hybridisation conditions with the primary probe,

(ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and

(iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.

Proposed Count 2

A method for the detection of a target nucleotide sequence, comprising:

(a) contacting the target nucleotide under conditions that permit hybridization with

(i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and

(ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and

(b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.

Proposed Count 3

A hybridization assay kit for the detection of a target nucleotide sequence, comprising:

(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and

(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.

Proposed Count 4

A nucleic acid hybridization assay wherein:

I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:

(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and

(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide,wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;

II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;

III. unbound multimer is removed;

IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;

V. unbound labeled oligonucleotide is removed; and

VI. the presence of label bound to the multimer is detected.

APPENDIX 3

COMPARISON OF COUNTS WITH REPRESENTATIVE PATENT CLAIMS

Count 3 (Claim 25 of US4882269)	Claim 49 of US4882269
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.	A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe cassette which comprises a cloning vector having (i) a multiple cloning site into which a target nucleotide sequence can be inserted and cloned and (ii) nucleotide sequences which are capable of hybridizing to their complements which comprise a plurality of secondary probes; and (b) the plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a nucleotide sequence capable of hybridizing to a different portion of the portion of the primary probe described in (a)(ii), which provides for the generation of an amplified signal when the plurality of secondary probes are hybridized to different portions of the portion of the primary probe described in (a)(ii).
Count 3 (Claim 25 of US4882269)	**Claim 1 of US5424188**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of	A hybridization assay kit for the detection of a target nucleotide sequence in a sample which target is hybridized to a primary probe, which primary probe has (1) a polynucleotide sequence complementary to the target nucleotide sequence and (2) a polymeric tail with a plurality of binding sites, each site incapable of binding to the target sequence and capable of binding a member of a family of secondary probes, which kit comprises: a plurality of secondary probes comprising a family of signal-generating probes, each member of the family having at least (1) a signal-generating

secondary probes are bound to different portions of the primary probe tail.	component and (2) a polymer capable of binding to a distinct binding site of the tail of the primary probe which site is not bound by other members of the family; which kit provides for the generation of an amplified signal when the plurality of secondary probes are bound to distinct binding sites of the tail of the primary probe
Count 4 (Claim 53 of US5124246)	**Claim 39 of US5124246**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	A synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds.

APPENDIX 4

COMPARISON OF COUNTS WITH APPLICATION CLAIMS

Count 1 (Claim 1 of the US4716106)	Claims 443 and 532-35 of Pergolizzi Application
A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of (a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and (b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of (i) contacting the sample under hybridisation conditions with the primary probe, (ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and (iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.	443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising: providing a composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal; forming a complex comprising said composition and said analyte; and detecting said analyte by a signal provided by said signal generating portion or portions present in said complex. 532. The process of claim 443, wherein said analyte is a DNA sequence, said bridging entity is a single-stranded DNA sequence, and said signalling entities are single-stranded DNA sequences. 533. The process of claim 532, wherein said bridging entity is derived from a filamentous phage. 534. The process of claim 533, wherein said signalling entities are derived from filamentous phages. 535. The process of claim 534, wherein said bridging entity codes for a gene product or

	fragment thereof, and said forming step comprises either (i) contacting said analyte with said bridging entity to form a first complex and thereafter contacting said first complex with said signalling entities to form said detectable complex or (ii) contacting said bridging entity with said signalling entities to form a first complex and thereafter contacting said first complex with said analyte to form said detectable complex.
Count 2 (Claim 1 of the US4882269)	**Claims 443 and 536-37 of Pergolizzi Application**
A method for the detection of a target nucleotide sequence, comprising: (a) contacting the target nucleotide under conditions that permit hybridization with (i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and (ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and (b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.	443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising: providing a composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal; forming a complex comprising said composition and said analyte; and detecting said analyte by a signal provided by said signal generating portion or portions present in said complex. 536. The process of claim 443, wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one

	or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte. 537. The process of claim 536, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.
Count 3 (Claim 25 of the US4882269)	**Claims 411 and 538-39 of Pergolizzi Application**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.	411. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof: (i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and (ii) a container carrying more than one non-radioactive signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequence or segment, and one or more signal generating portions, each such portion being capable of providing a detectable signal. 538. The kit of claim 411, wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte. 539. The kit of claim 538, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.
Count 4 (Claim 53 of the US5124246)	**Claims 443 and 540-41 of Pergolizzi Application**
A nucleic acid hybridization assay	443. A process for detecting an analyte

wherein:

I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:

(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and

(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;

II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;

III. unbound multimer is removed;

IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;

V. unbound labeled oligonucleotide is removed; and

VI. the presence of label bound to the multimer is detected.

having one or more molecularly recognizable portions thereon, comprising:

providing a composition of matter comprising:

a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and

a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal;

forming a complex comprising said composition and said analyte; and

detecting said analyte by a signal provided by said signal generating portion or portions present in said complex.

540. The process of claim 443, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.

541. The process of claim 540, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences, said bridging entity first portion is capable of encoding a gene product or fragment thereof, and the process further comprises one or more washing steps prior to detection.

Count 4 (Claim 53 of the US5124246)	Claims 283 and 542-43 of Pergolizzi Application
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	283. A composition of matter comprising: a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and a second part which comprises one or more non-radioactive signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal. 542. The composition of claim 283, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments. 543. The composition of claim 542, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences and said bridging entity first portion is capable of encoding a gene product or fragment thereof.
Count 4 (Claim 53 of the US5124246)	**Claims 411 and 544-45 of Pergolizzi Application**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in	411. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof: (i) a container carrying a molecular

an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and (ii) a container carrying more than one non-radioactive signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequences or segments, and one or more signal generating portions, each such portion being capable of providing a detectable signal. 544. The kit of claim 411, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments. 545. The kit of claim 544, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences and said bridging entity first portion is capable of encoding a gene product or fragment thereof.

APPENDIX 5

SUPPORT FOR NEW APPLICATION CLAIMS

New Pergolizzi Process Claims 532-35	Support in Pergolizzi Application
532. The process of claim 443, wherein *said analyte is a DNA sequence, **said bridging entity is a single-stranded DNA sequence, and ***said signalling entities are single-stranded DNA sequences.	*Original Claims 4, 7, 69, 102 and 147 (as filed May 5, 1983). Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2. **Original Claims 7, 20, 29, 30, 69, 125 and 147. Pergolizzi Specification p.10, 2nd para.; p.17, 2nd para.; pp.57-58; Fig.2. ***Original Claims 43 and 134. Pergolizzi Specification pp.57-58; Fig.2.
533. The process of claim 532, wherein said bridging entity is derived from a filamentous phage.	Original Claims 31, 32, 70, 111, 126-27 and 148. Pergolizzi Specification p.16, 2nd para; pp.57-61.
534. The process of claim 533, wherein said signalling entities are derived from filamentous phages.	Original Claims 39, 44, 45, 67, 71, 135-36 and 149. Pergolizzi Specification p.16, 2nd para; pp.57-61.
535. The process of claim 534, wherein *said bridging entity codes for a gene product or fragment thereof, and **said forming step comprises either (i) contacting said analyte with said bridging entity to form a first complex and thereafter contacting said first complex with said signalling entities to form said detectable complex or (ii) contacting said bridging entity with said signalling entities to form a first complex and thereafter contacting said first complex with said analyte to form said detectable complex.	*Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.; p.17, 2nd para.; p.30, 2nd and 3rd paras. **Pergolizzi Specification p.28, 2nd para.; p.31 4th para. to p.34, 1st para.; Figs.1-2.
New Pergolizzi Process Claims 536-37	**Support in Pergolizzi Application**
536. The process of claim 443, wherein *said analyte is a polynucleotide,	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th

**said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and ***and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.	para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2. **Original Claims 18 and 113. Pergolizzi Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2. ***Pergolizzi Specification p.14, 2nd para; Fig.2.
537. The process of claim 536, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.	Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.
New Pergolizzi Kit Claims 538-39	**Support in Pergolizzi Application**
538. The kit of claim 411, wherein *said analyte is a polynucleotide, **said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and ***and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2. **Original Claims 18 and 113. Pergolizzi Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2. ***Pergolizzi Specification p.14, 2nd para; Fig.2.
539. The kit of claim 538, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.	Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.; p.22, 1st para.
New Pergolizzi Method Claims 540-41	**Support in Pergolizzi Application**
540. The process of claim 443, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support, **said bridging entity comprises non-naturally occurring or artificially modified DNA, ***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, ****said bridging entity first portion is	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para. **Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para. ***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para.. ****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23,

covalently bound to said bridging entity second portion, and *****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.	1st para.; p.25, 3rd para.; Fig.2. *****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.
541. The process of claim 540, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences, **said bridging entity first portion is capable of encoding a gene product or fragment thereof, and ***the process further comprises one or more washing steps prior to detection.	*Original Claims 43 and 134. Pergolizzi Specification pp.57-58. **Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para. ***Pergolizzi Specification p.18, 1st para.; p.28, 2nd para.
New Pergolizzi Composition Claims 542-43	**Support in Pergolizzi Application**
542. The composition of claim 283, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support, **said bridging entity comprises non-naturally occurring or artificially modified DNA, ***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, ****said bridging entity first portion is covalently bound to said bridging entity second portion, and *****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para. **Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para. ***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para.. ****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2. *****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.
543. The composition of claim 542, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences and	*Original Claims 43 and 134. Pergolizzi Specification pp.57-58. **Original Claims 16 and 111. Pergolizzi

**said bridging entity first portion is capable of encoding a gene product or fragment thereof.	Specification p.14, 3rd para.
New Pergolizzi Kit Claims 544-45	**Support in Pergolizzi Application**
544. The kit of claim 411, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support, **said bridging entity comprises non-naturally occurring or artificially modified DNA, ***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, ****said bridging entity first portion is covalently bound to said bridging entity second portion, and *****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.	*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para. **Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para. ***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para.. ****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2. *****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.
545. The kit of claim 544, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences and **said bridging entity first portion is capable of encoding a gene product or fragment thereof.	*Original Claims 43 and 134. Pergolizzi Specification pp.57-58. **Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.

APPENDIX 6

APPLICATION CLAIMS PENDING BEFORE

DECEMBER 29, 1988, NOVEMBER 21, 1990, OR JUNE 23, 1993

Claim 1 of US4716106 (Count 1)	Claims 1 and 69-71 of Original Pergolizzi Application
A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of (a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and (b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of (i) contacting the sample under hybridisation conditions with the primary probe, (ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and (iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.	1. A method of detecting in a sample an analyte (A) having a molecularly recognizable portion thereon, which comprises: providing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable portion on said analyte; and (ii) a portion comprising a polynucleotide sequence; and (C) a signalling entity having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity, thereby to form a stable polynucleotide hybrid, and (ii) a signal generating portion; forming a complex comprising: (1) said analyte (A) complexed through said molecularly recognizable portion to (2) said recognizing portion of said entity (B); said entity (B) being complexed through said polynucleotide portion thereon to (3) said polynucleotide portion of said signalling entity (C); and detecting a signal by means of said signal generating portion present in said complex. 69. The method of Claim 1 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said step of detection by means of said signal generating

	portion on said signalling entity is based on non-radioactive detection. 70. The method of Claim 69 wherein said bridging entity is derived from a filamentous phage. 71. The method of Claim 69 wherein said signalling entity is derived from a filamentous phage.
Claim 1 of US4716106 (Count 1)	**Claims 154-56, 159, 161, 165 and 168 of Pergolizzi's Amendment of June 3, 1985**
A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of (a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and (b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of (i) contacting the sample under hybridisation conditions with the primary probe, (ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and (iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.	154. A method of detecting in a sample an analyte having a molecularly recognizable portion thereon, comprising: forming a detectable complex comprising (a) said analyte, (b) a non-naturally-occurring molecular bridging entity comprising a portion capable of recognizing and complexing to said molecularly recognizable analyte portion, and a portion comprising a polynucleotide sequence, and (c) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion and a signal generating portion; and detecting said analyte by a signal provided by said signal generating portion present in said detectable complex. 155. The method according to claim 154, characterized in that said forming step comprises contacting said analyte with said bridging entity to form a first complex and contacting said first complex with said signalling entity to form said detectable complex. 156. The method according to claim 154, characterized in that said forming step comprises contacting said bridging entity with said signalling entity to form a first complex and contacting said first complex with said analyte to form said detectable complex. 159. The method according to claim 158, characterized in that said molecularly recognizable portion on said analyte is selected from the group consisting of an RNA or DNA

	oligo- or polynucleotide sequence, and a protein. 161. The method according to claim 154, characterized in that said bridging entity recognizing portion is an RNA or DNA oligo- or polynucleotide sequence. 165. The method according to claim 154, characterized in that said bridging entity is selected from the group consisting of a single-stranded, double stranded or partially double-stranded circular DNA polymer, a circular DNA polymer derived from a filamentous phage, an M13 phage or a variant thereof. 168. The method according to claim 154, characterized in that said signalling entity is selected from the group consisting of a single stranded, double stranded, or partially double-stranded polynucleotide polymer, a naturally occurring modified DNA, a polynucleotide polymer derived from a T (even) phage, a modified DNA carrying a cloned insert, a polymer derived from a filamentous phage, M13 phage or a variant thereof, and a polymer derived from a circular DNA molecule covalently attached to a non radiolabelled signal generating portion.
Claim 1 of US4882269 (Count 2)	**Claims 154 and 161-65 of Pergolizzi's Amendment of June 3, 1985**
A method for the detection of a target nucleotide sequence, comprising: (a) contacting the target nucleotide under conditions that permit hybridization with (i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and (ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and (b) detecting the amplified signal	154. A method of detecting in a sample an analyte having a molecularly recognizable portion thereon, comprising: forming a detectable complex comprising (a) said analyte, (b) a non-naturally-occurring molecular bridging entity comprising a portion capable of recognizing and complexing to said molecularly recognizable analyte portion, and a portion comprising a polynucleotide sequence, and (c) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion and a signal generating portion; and detecting said analyte by a signal provided by said signal generating portion

generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.	present in said detectable complex. 159. The method according to claim 158, characterized in that said molecularly recognizable portion on said analyte is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence, and a protein. 161. The method according to claim 154, characterized in that said bridging entity recognizing portion is an RNA or DNA oligo- or polynucleotide sequence. 162. The method according to claim 154, characterized in that said bridging entity polynucleotide portion is a polynucleotide sequence of low complexity. 163. The method according to claim 162, characterized in that said bridging entity polynucleotide sequence is selected from the group consisting of a polydeoxy G, polydeoxy C, polydeoxy T or polydeoxy A. 164. The method according to claim 161, characterized in that said bridging entity polynucleotide portion and said bridging entity recognizing portion are not capable of hybridizing to identical oligo- and polynucleotide sequences. 165. The method according to claim 154, characterized in that said bridging entity is selected from the group consisting of a single-stranded, double stranded or partially double-stranded circular DNA polymer, a circular DNA polymer derived from a filamentous phage, an M13 phage or a variant thereof.
Claim 25 of US4882269 (Count 3)	**Claims 100, 102 and 147-48 of Original Pergolizzi Application**
A hybridization assay kit for the detection of a target nucleotide sequence, comprising: (a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and (b) a plurality of secondary probes comprising a family of signal-generating probes	100. A kit useful for the detection of an analyte (A) having a molecularly recognizable portion thereon, comprising; I) a carrier being compartmentalized to receive in close confinement therein one or more container means; II) a first container means containing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable

each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.	portion on said analyte (A); and (ii) a portion comprising a polynucleotide sequence; and (III) a second container means containing a signalling entity (C) having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity (B) thereby by form a stable polynucleotide hybrid; and (ii) a signal generating portion. 102. The kit of Claim 100 wherein said recognizing portion on said bridging entity comprises a polynucleotide sequence. 147. The kit of Claim 100 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said signal generating portion on said signalling entity is based on non-radioactive detection. 148. The kit of Claim 147 wherein said bridging entity is derived from a filamentous phage.
Claim 53 of US5124246 (Count 4)	**Claims 1, 4, 7, 16, 20 and 69 of Original Pergolizzi Application**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled	1. A method of detecting in a sample an analyte (A) having a molecularly recognizable portion thereon, which comprises: providing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable portion on said analyte; and (ii) a portion comprising a polynucleotide sequence; and (C) a signalling entity having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity, thereby to form a stable polynucleotide hybrid, and (ii) a signal generating portion;

oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	forming a complex comprising: (1) said analyte (A) complexed through said molecularly recognizable portion to (2) said recognizing portion of said entity (B); said entity (B) being complexed through said polynucleotide portion thereon to (3) said polynucleotide portion of said signalling entity (C); and detecting a signal by means of said signal generating portion present in said complex. 4. The method of Claim 1 wherein the molecularly recognizable portion on said analyte comprises nucleic acid. 7. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises a polynucleotide sequence. 16. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity codes for a gene product or fragment thereof. 20. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to another polynucleotide sequence. 69. The method of Claim 1 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said step of detection by means of said signal generating portion on said signalling entity is based on non-radioactive detection.
Claim 53 of US5124246 (Count 4)	**Claims 172, 173 and 179 of Pergolizzi's Amendment of June 3, 1985**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of	172. A kit useful for the detection in a sample of an analyte having a molecularly recognizable portion thereon, comprising as components thereof: (i) a non-naturally occurring molecular bridging entity comprising a portion capable of recognizing said molecularly recognizable portion on said analyte; and a portion comprising a polynucleotide sequence; and

hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	(ii) a signalling entity comprising a polynucleotide portion capable of annealling to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion, and a signal generating portion. 173. The kit according to claim 172, characterized in that said bridging entity recognizing portion is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence, an antigen, an antibody, a saccharide, a lectin, a hormone, a receptor, an enzyme inhibitor, a cofactor bonding site, an enzyme active site, and a receptor protein. 179. The kit according to claim 172, characterized in that said signalling entity is selected from the group consisting of a single-stranded, double-stranded or partially double-stranded polynucleotide polymer, a naturally-occurring modified DNA, a polynucleotide polymer derived from a T (even) phage, a modified DNA carrying a cloned insert, a polymer derived from a filamentous phage, M13 phage or a variant thereof, and a polymer derived from a circular DNA molecule covalently attached to a non-radiolabelled signal generating portion.
Count 4 (Claim 53 of US5124246)	**Claim 193 of Pergolizzi's Amendment of Oct. 9, 1990**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of	193. A kit for the detection in a sample of an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising as components thereof: (i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable portion on said analyte; and as second portion comprising more than one polynucleotide sequence; and (ii) a container carrying a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity second polynucleotide portion, and a signal generating

interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	portion; which molecular bridging entity and signalling entity form a detectable complex with said analyte, wherein the at least one component of said detectable complex is immobilized or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portion thereof.
Claim 53 of US5124246 (Count 4)	**Claim 196 of Pergolizzi's Amendment of Oct. 9, 1990**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte	196. A method of detecting in a sample an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising: forming a detectable complex comprising (a) said analyte, (b) a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable analyte portion, and a second portion comprising more than one polynucleotide sequence, and (c) more than one signalling entity, each said signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with the polynucleotide sequences in said bridging entity second portion and a signal generating portion; and detecting said analyte by an amplified signal provided by said signal generating portions present in said detectable complex, wherein at least one component of said detectable complex is immobilized or the incorporation of the analyte into the detectable complex causes a

nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is removed; and VI. the presence of label bound to the multimer is detected.	detectable change in the signal generating portions thereof.
Claim 53 of US5124246 (Count 4)	**Claims 194, 199, 209-213, 217-18 and 220 of Pergolizzi's Amendment of Dec. 22, 1992**
A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds; II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte; III. unbound multimer is removed; IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units; V. unbound labeled oligonucleotide is	194. A composition of matter comprising: a molecular bridging entity comprising a first portion capable of recognizing and binding to a molecularly recognizable portion on an analyte, and a second portion comprising a nucleic acid; and a universal signalling entity comprising a nucleic acid portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity nucleic acid second portion, and a signal generating portion capable of providing, directly or indirectly, a detectable signal. 199. The composition according to claim 194, wherein said analyte is selected from the group consisting of a nucleic acid and a protein. 209. The composition according to claim 194, wherein said molecular bridging recognizing first portion comprises a nucleic acid. 210. The composition according to claim 209, wherein said nucleic acid comprises an oligo- or polynucleotide. 211. The composition according to claim 210, wherein said oligo- or polynucleotide comprises a modified oligo- or polynucleotide. 212. The composition according to claim 210, wherein said oligo- or polynucleotide is single-stranded or partially double-stranded. 213. The composition according to claim 210, wherein said oligo- or polynucleotide is circular or linear.

removed; and VI. the presence of label bound to the multimer is detected.	217. The composition according to claim 194, wherein said polynucleotide sequence in the molecular bridging entity second portion is linear or circular. 218. The composition according to claim 194, wherein said nucleic acid in the molecular bridging entity second portion is single-stranded or partially double-stranded. 220. The composition according to claim 194, wherein said polynucleotide sequence in the molecular bridging entity second portion is derived from a phage selected from the group consisting of a T even phage, a filamentous phage, an M13 phage, or a variant thereof.

In response to the Notice, Applicants are filing a Communication to submit the missing Figure 2. A copy of the Communication with Figure 2 is attached to this Petition as Exhibit B. It is believed that the Rule 1.60 filing for the application is now complete. Any inconvenience to the Patent Office and the Applications Branch caused by the inadvertently missing figure is sincerely regretted.

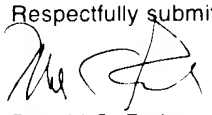
In view of the accompanying Communication (Exhibit B), it is respectfully requested that the subject application be accorded its June 7, 1995 filing date.

The fee for filing this Petition Under 37 C.F.R. §1.182 is $130.00. The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for the requisite fee of $130.00 set forth in 37 C.F.R. §1.17(h). The Patent and Trademark Office is further authorized hereby to charge Deposit Account No. 05-1135 for any other fees required in connection with this Petition or the accompanying Communication, and to credit any overpayment thereto.

A duplicate copy of this Petition but without attached Exhibits A and B is also submitted herewith.

Favorable action on this Petition is earnestly solicited.

Respectfully submitted,



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